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A Cytological Analysis of 24 Taxa in *Aristolochia* Subgenera *Siphisia* and *Aristolochia* (Aristolochiaceae)

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Cytological investigations were carried out on 24 taxa representing *Aristolochia* subgenera *Siphisia* and *Aristolochia*. Of them, 17 taxa (16 species and one variety) were examined for the first time. In subgeneus *Siphisia*, all the eight species, *A. austoyunnanensis*, *A. kwangsiensis*, *A. macrophylla*, *A. moupiensis*, *A. nakaoi*, *A. paracleta*, *A. salvadorensis* and *A. tricaudata* show the same number of $2n=32$. While in subgenus *Aristolochia* three different chromosome numbers were observed: $2n=16$ in *A. maxima* and *A. ovalifolia*; $2n=14$ in *A. arcuata*, *A. argentiana*, *A. clematitis*, *A. cymbifera* var. *abbreviata*, *A. fimbriata*, *A. goliathiana*, *A. macroura*, *A. micrantha*, *A. odoratissima*, *A. ringens* and *A. tiangularis*; $2n=12$ in *A. jackii*, *A. kankauensis* and *A. tagala*. The chromosome numbers obtained are relatively well in congruence with the classification system and with the molecular phylogeny. Based on the present and previous studies the subgenus *Siphisia* is characterized by the number $2n=32$. In contrast, the subgenus *Aristolochia* is diversified in chromosome number, showing the three different numbers. Subsection *Podanthemum* and series *Thyrsicae* seem to be characterized by the numbers, $2n=12$ and $2n=16$, respectively.

Key words: *Aristolochia*, chromosome number, *Siphisia*, systematics, phylogeny

Aristolochia L. is a large genus comprising more than 400 species, most of them twining lianas, and is widely distributed in tropical and subtropical regions almost all over the world (Schmidt 1935, Pfeifer 1966, Ma 1989, Gonzalez 1999). In the most current classification system of Gonzalez (1999) and Gonzalez & Stevenson (2000), the genus was subdivided into three subgenera, *Siphisia* (Raf.) Duchartre, *Aristolochia* and *Pararistolochia* Hutch & Dalz., based on floral morphology such as the shape of perianth tube, the number of styles and anthers on a gynostemium, the pattern of perianth development, and on the mode of capsule dehiscence and the inflorescence architecture. Although the phylogenetic relationships among the three subgenera are still unclear, monophyly of the subgenera *Siphisa* and *Aristolochia* has been just clarified on the basis of *matK* gene sequences (Murata *et al.* 2001). In the earlier taxonomic papers by Ma (1989, 1990) the subgenera *Siphisia* and *Aristolochia* were cytologically characterized by tetraploid number $2n=28$ and diploid number

$2n$=14 (rarely $2n$=12), respectively. However, our previous study (Sugawara & Murata 1992) and the latest karyological information (Yu *et al.* 1995) have provided that different chromosome numbers which have not been reported so far are found in some species of the genus *Aristolochia*,

TABLE 1.Species examined of *Aristolochia*, and their collections and chromosome numbers. Systematics of *Aristolochia* is from Gonzalez (1999). Nomenclature for the classification system is altered according to International Code of Botanical Nomenclature (Saint Louis Code).

Species	Collection data and voucher specimen	Chromosome number (2n)	
		Present count (N)*	Previous reports
Aristolochia L.			
Subgen. *Siphisia* (Raf.) Duchartre (consist. approximately 50 species, from North and Central America, and temperate and tropical Asia)			
A. austroyunnanensis S.M. Hwang	Guangxi, China (cult.), SETS15	32 (1)	
A. kwangsiensis C.F. Liang	Guangxi, China (cult.), SETS28	32 (1)	32 (Yu *et al.*, 1995)
A. macrophylla Lam.	U.S.A. (cult.), SETS3	32 (1)	
A. moupinensis Franch.	Sichuan, China (cult.), SETS52	32 (1)	
A. nakaoi F. Maekawa.	Nepal (cult.), SETS14	32 (1)	
A. paracleta Pfeifer	Mt. Uyuca, Honduras (cult.), J. Murata *et al.* 97-206	32 (1)	
A. salvadorensis Standl.	Guatemala (cult.), J. Murata 00-3	32 (1)	
A. tricaudata Lem.	Mexico (cult.), SETS42	32 (1)	
Subgen. *Aristolochia*			
Sect. *Aristolochia* (consist. approximately 120 species, from Europe, North and East Africa, Asia and North Australia)			
Subsect. *Aristolochia*			
A. clematitis L.	Europe (cult.), SETS17	14 (1)	14 (Fedrov, 1969; Nardi, 1984)
A. goliathiana M. J. Parsons	South Kalimantan (cult.), SETS19	14 (1)	
Subsect. *Podanthemum* (Klotzsch) Schmidt			
A. jackii Steud.	Madagascar (cult.), SETS56	12 (1)	
A. kankauensis Sasaki	Hengchun, Taiwan (cult.), SETS35	12 (1)	
A. tagala Cham.	Nirgili, India (cult.), SETS48	12 (1)	14 (Gregory, 1956)
A. tagala Cham.	New Guinea (cult.), SETS27	12 (1)	14 (Gregory, 1956)
Sect. *Gymnolobus* Duchartre (includ. ca. 210 species, from the New World)			
Subsect. *Hexandrae* Duchartre			
Ser. *Thyrsicae* F. Gonzalez			
A. maxima L.	South America (cult.), SETS37	16 (1)	
A. ovalifolia Duchartre	Mt. El. Chile, Honduras (cult.), SETS66	16 (1)	
Ser. *Hexandrae*			
A. arcuata Mast.	cult., SETS29	14 (1)	14 (Gregory, 1956)
A. argentina Griseb.	cult., SETS33	14 (1)	
A. cymbifera Mart. & Zucc. var. *abbreviata* Duchartre	cult., SETS55	14 (1)	14 (Gregory, 1956)
A. *fimbriata* Cham. & Schltdl.	South America (cult.), SETS11	14 (1)	14 (Gregory , 1956)
A. *macroura* Duchartre	cult., SETS23	14 (1)	14 (Moore, 1970)
A. *odoratissima* L.	cult., SETS31	14 (1)	
A. *ringens* Vahl	cult., SETS57	14 (1)	14 (Gregory , 1956), 12 (Yu *et al.*, 1995)
A. *triangularis* Cham.	cult., SETS20	14 (1)	
Subsect. *Pentandrae* Duchartre			
A. *micrantha* Duchartre	Mexico (cult), SET78	14 (1)	

cult., cultivated in Medicinal Plant Garden, Setsunan University

*, number of plants examined.

particularly of the subgenus *Siphisia* occurring in eastern Asia. We wonder what evolutionary diversification of chromosome numbers has occurred within the genus.

As mentioned above, earlier cytological studies of *Aristolochia* have supplied valuable data for elucidating the relationships among the species. Nevertheless, our knowledge on cytological features of the genus is still limited to approximately 50 species distributed mainly in eastern Asia, North America and Europe. We extended our cytological investigations to the species distributed in tropical and subtropical regions of the world. In this study we cytologically investigate 24 taxa of *Aristolochia* representing the subgenera *Siphisia* and *Aristolochia*. Our goals are to present cytological data for the 24 taxa of *Aristolochia* and to examine the implications of our results with the molecular phylogeny just presented and with the current classification system of *Aristolochia* subgenera *Siphisia* and *Aristolochia*.

Materials and Methods

Most of the plant materials examined are comparable to those used for analysis of *matK* gene sequences by Murata *et al.* (2001). These materials were collected from natural populations or from cultivated plants, and most of them were transplanted in pots at the Medicinal Garden, Faculty of Pharmaceutical Science, Setsunan University, Osaka Prefecture. In total we examined 24 taxa (23 species and one variety) of *Aristolochia*, seven of them representing the subgenus *Siphisia* (Raf.) Duchartre and the others assigned to the subgenus *Aristolochia*. Their systematic arrangements fundamentally follow Gonzalez (1999) and Gonzalez & Stevenson (2000), and their collection data and voucher specimens are presented in Table 1. The voucher specimens are deposited in the Herbarium of the University of Tokyo (TI).

Cytological analysis was made by the following procedures. Root tips were pretreated with 0.002M-hydoroxyquinoline solution for 2.5-3 hr at room temperature and fixed with 45% acetic acid solution for 20-25min. After being macerated in a 1:2 mixture of 45% acetic acid and 1N HCl for 30 sec at 60°C, the root tips were stained with 1 % acetic orcein solution for overnight. Subsequently they were squashed in the standard way. Nomenclature for centromeric position follows Levan *et al.* (1964).

Molecular phylogeny used in the present study fundamentally follows that of Murata *et al.* (2001), in which information on seven species are newly added. The seven species added and their voucher specimens and gene bank accession numbers are provided in Appendix 1.

Results

Somatic chromosome numbers obtained from 24 taxa representing *Aristolochia* subgen. *Siphisia* and *Aristolochia* are summarized in Table 1. As can be seen from Table 1, four different chromosome numbers were found among the taxa concerned. The somatic chromosome numbers and karyological features are described below.

Somatic chromosome number $2n=32$ was found in *A. austroyunnanensis* S. M. Hwang, *A. kwangsiensis* C. F. Liang, *A. macrophylla* Lam., *A. moupiensis* Franch., *A. nakaoi* F. Maekawa, *A. paracleta* Pfeifer, *A. salvadorensis* Standl. and *A. tricaudata* Lem. The chromosome number of *A. kwangsiensis* was in agreement with a previous report (Yu *et al.* 1995). Among the species we could find no significant differences in karyotype: their metaphase chromosomes were

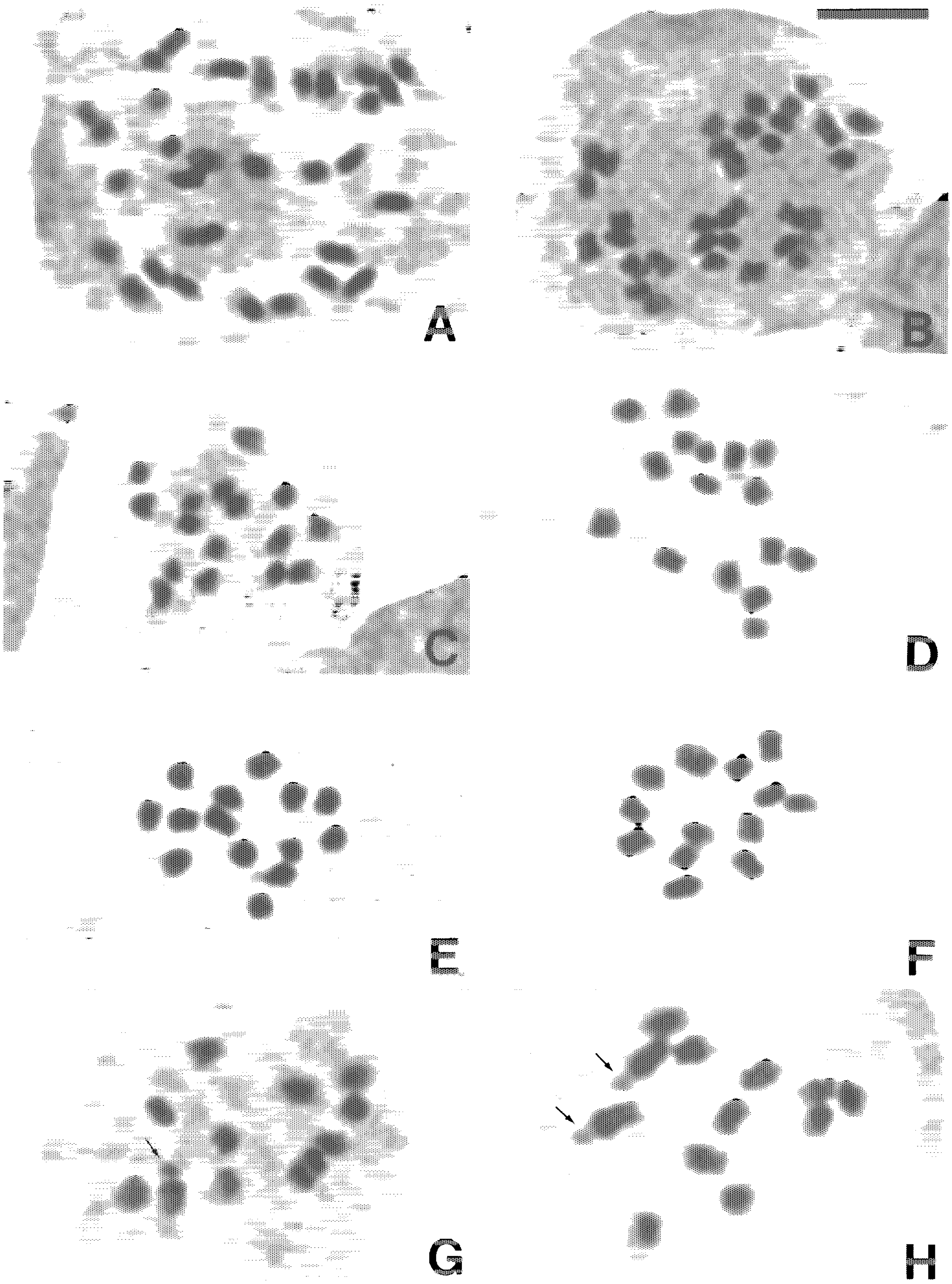

FIG. 1. Somatic metaphase chromosomes of some representatives in *Aristolochia*. A: *A. nakaoi* ($2n=32$). B: *A. tricaudata* ($2n=32$). C: *A. maxima* ($2n=16$). D: *A. ovalifolia* ($2n=16$). E: *A. argentina* ($2n=14$). F: *A. cymbifera* var. *abbreviata* ($2n=14$). G: *A. jackii* ($2n=12$). H: *A. tagala* ($2n=12$). Arrows indicate a chromosome with secondary constriction. Scale bar represents 5μm.

monomodal and usually varied in length from about 1.5 μm to 0.5 μm, having a centromere at median or submedian positions (Fig. 1A, B).

Somatic chromosome number 2*n*=16 was found in *A. maxima* L. and *A. ovalifolia* Duchartre. The two species are similar to each other in having a monomodal karyotype. Their metaphase chromosomes usually varied in length from about 1.2 μm to 0.6 μm and had a centromere at median or submedian positions (Fig. 1C, D).

Somatic chromosome number 2*n*=14 was found in *A. clematitis* L., *A. goliathiana* M. J. Parsons, *A. arcuata* Mast., *A. argentina* Griseb., *A. cymbifera* Mart. & Zucc. var. *abbreviata* Duchartre, *A. fimbriata* Cham., *A. macroura* Gomez, *A. micrantha* Duchartre, *A. odoratissima* L., *A. ringens* Vahl and *A. triangularis* Cham. Previous reports (Gregory 1956, Fedrov 1969, Moore 1970, Nardi 1984) for *A. clematitis, A. arcuata, A. macroura* and *A. fimbriata* were confirmed in the present study. Although the two different numbers 2*n*=14 (Gregory 1956) and 2*n*=12 (Yu *et al.* 1984) had been reported for *A. ringens*, we determined the number 2*n*=14 for the species. Among the species no significant differences were found in karyotype: their metaphase chromosomes were monomodal and usually varied in length from 1.5μm to 0.8μm, having a centromere at median or submedian positions (Fig. 1E, F).

Somatic chromosome number 2*n*=12 was found in *A. jackii* Steud., *A. kankauensis* Sasaki and *A. tagala* Cham. We could find no significant differences in karyotype among the three species: their metaphase chromosomes were monomodal and usually varied in length from about 2.0μm to 1.0μm, having a centromere at median or submedian positions (Fig. 1G, H). The two longest chromosomes with submedian centromeres had a secondary constriction on their long arm, and then were obviously distinguished from the others. Previously, the number 2*n*=14 was reported for *A. tagala* (Gregory 1956). In this study, however, we have determined the number 2*n* =12 for the species.

Discussion

In the present study we determined the chromosome numbers of 24 taxa representing *Aristolochia* subgenera *Siphisia* and *Aristolochia*, 17 taxa of which were examined for the first time. Based on the results obtained we discuss on an implication of the chromosome numbers with the current molecular phylogeny by Murata *et al.* (2001) as well as with the classification system of *Aristolochia* by Gonzalez (1999) and Gonzalez & Stevenson (2000).

As shown in Figure 2, the phylogenetic relationship of the subgenera *Siphisia* and *Aristolochia* has been just clarified on the basis of *matK* gene sequences by Murata *et al.* (2001). The chromosome numbers obtained from the present and previous studies (Gregory 1956, Fedrov 1969, Moore 1970, Nardi 1984, Sugawara & Murata 1992, Yu *et al.* 1995) and the current classification system of the two subgenera (Gonzalez 1999) are also shown in the figure. It can be seen from this figure that the chromosome numbers are relatively well in congruence with the molecular phylogeny as well as with the classification system, and that the subgenus *Siphisia* is consistent in chromosome number while the subgenus *Aristolochia* presents a diversification of chromosome numbers.

The subgenus *Siphisia* is morphologically characterized by having a basally dehiscent capsule, a tri-lobed perianth tube, and a tri-lobed gynostemium (Gonzalez 1999). Monophyly of the subgenus *Siphisa* has been

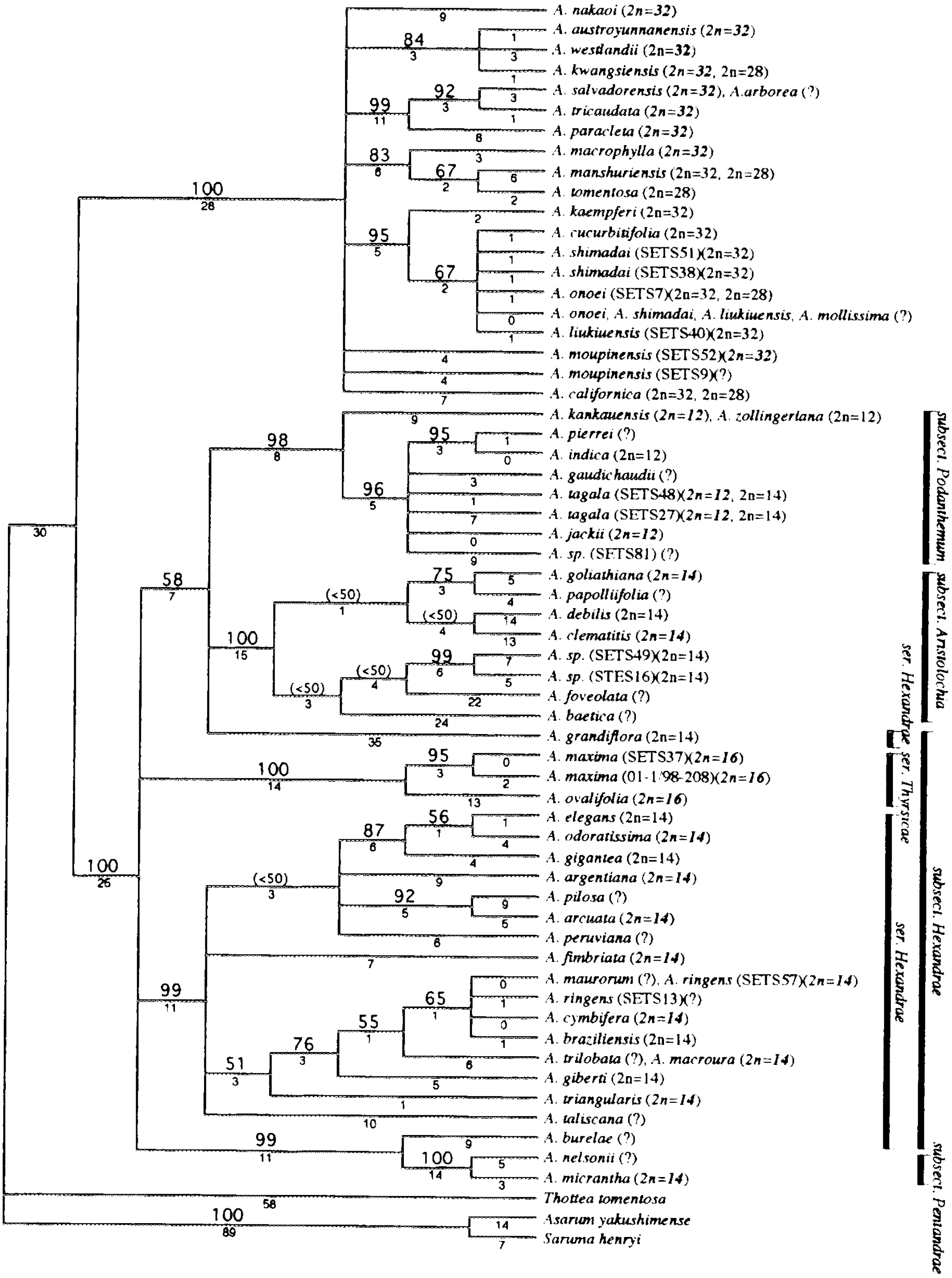

FIG. 2. Implication of the chromosome numbers with the molecular phylogeny in *Aristolochia* subgenera *Siphisia* and *Aristolochia*. The molecular phyogenetic tree represents a strict consensus tree of 14,032 equally parsimonious ones for the genus *Aristolochia* based on the *matK* gene sequences. Length=688steps, CI=0.680, RI=0.883, RC=0.601. A branch length is given below each node. A percentage of the 1,000 bootstrap values (>50%) is also given above each node. This molecular phylogenetic tree fundamentally follows Murata *et al.* (2001) with slight modification in which eight taxa are newly added. The classification system of subgenera *Siphisia* and *Aristolochia* (Gonzalez 1999) is indicated on the right margin. The chromosome numbers of the species concerned are from the present (printed in italic) and previous (printed in roman) studies (see also Table 2).

TABLE 2. Chromosome numbers of 68 taxa of *Aristolochia* determined so far and their systematic arrangements.

Species	Chromosome number	Reference
Aristolochia L.		
Subgen. *Siphisia* (Raf.) Duchartre		
A. austroszechuanica C.Y. Cheng et J. L. Wu	2n = 32	Yu *et al.* (1995)
A. austroyunnanensis S.M. Hwang	2n = 32	Present authors
A. californica Torr.	2n = 28	Gregory (1956)
	2n = 32	Ornduff (1967)
A. compressicaulis Z.L. Yang	2n = 28	Ma (1989)
	2n = 32	Yu *et al.* (1995)
A. convolvulacea Small	2n = 28	Gregory (1956)
A. cucurbitifolia Small	2n = 32	Sugawara & Murata (1992)
A. jinshanensis Z.L. Yang et S.X. Tan	2n = 28	Ma (1989)
A. kaempferi Willd	2n = 32	Sugawara & Murata (1992)
A. kunmingensis C.Y. Cheng et J.S. Ma	2n = 28	Ma (1989)
	2n = 32	Yu *et al.* (1995)
A. kwangsiensis C.F. Liang	2n = 28	Ma (1989)
	2n = 32	Yu *et al.* (1995), Present authors
A. liukiuensis Hatushima	2n = 32	Sugawara & Murata (1992)
A. macrophylla Lam.	2n = 32	Present authors
A. manshuriensis Kom.	2n = 28	Gregory (1956), Goldblatt & Johnson (1991), Yu *et al.* (1995)
	2n = 32	Sugawara & Murata (1992)
A. mollis Dunn	2n = 32	Yu *et al.* (1995)
A. moupinensis Franch.	2n = 32	Present authors
A. nakaoi F. Maekawa.	2n = 32	Present authors
A. nashii Kearney	2n = 28	Gregory (1956)
A. onoei Franch. et Sav.	2n = 28	Huziwara (1962)
	2n = 32	Sugawara & Murata (1992)
A. paracleta Pfeifer	2n = 32	Present authors
A. salvadorensis Standl.	2n = 32	Present authors
A. serpentaria L.	2n = 28	Gregory (1956)
A. shimadai Hayata	2n = 32	Sugawara & Murata (1992)
A. sipho L'Herit	2n = 28	Gregory (1956)
A. tomentosa Sims	2n = 28	Gregory (1956)
A. tricaudata Lem.	2n = 32	Present authors
A. westlandii Hemsl.	2n = 32	Sugawara & Murata (1992)
Subgen. *Aristolochia*		
Sect. *Aristolochia*		
Subsect. *Aristolochia*		
A. bianorii Sennen et Pau	2n = 12	Goldblatt (1981)
A. clematitis L.	2n = 14	Fedrov (1969), Goldblatt (1981), Nardi (1984)
A. clusii Lojac.	2n = 12	Nardi (1984)
A. debilis Siebold e Zucc.	2n = 14	Ma (1989), Sugawara & Murata (1992), Yu *et al.* (1995)
A. elongata (Duchartre) Nardi	2n = 10	Goldblatt & Johnson (1991)
A. goliathiana M. J. Parsons	2n = 14	Present authors
A. guichardii P.H. Davis et Khan	2n = 10	Goldblatt & Johnson (1996)
A. haitiensis Ekman et O. Schmidt	2n = 14	Gregory (1956)
A. insularis Nardi et Arrigoni	2n = 12	Nardi (1984)
A. longa L.	2n = 28	Gregory (1956)
A. longilinqua C.Y. Cheng et W. Yu	2n = 14	Yu *et al.* (1995)
A. lutea Desf.	2n = 8	Nardi (1984)
A. navicularis Nardi	2n = 24	Nardi (1984)
A. pallida Willd.	2n = 8	Moore (1977)

TABLE 2. (continued)

	2n=10	Nardi (1984)
A. parvifolia Smith	2n=12	Goldblatt & Johnson (1991)
A. paucinervis Pomel	2n=36	Nardi (1984)
A. pistolochia L.	2n=14	Goldblatt & Johnson (1996)
A. rotunda L.	2n=12	Nardi (1984)
	2n=14	Gregory (1956)
A. sempervirens L.	2n=14	Goldblatt & Johnson (1996)
A. sicula Tineo	2n=16	Nardi (1984)
A. tubiflora Dunn	2n=14	Yu *et al.* (1995)
A. tyrrhena Nardi et Arrigoni	2n=26	Nardi (1984)
Subsect. *Podanthemum* (Klotzsch) Schmidt		
A. bracteata Retz.	2n=12	Fedrov (1969)
A. contorta Bunge	2n=14	Goldblatt & Johnson (1990)
	2n=12	Yu *et al.* (1995)
A. indica L.	2n=12	Fedrov (1969)
A. jackii Steud.	2n=12	Present authors
A. kankauensis Sasaki	2n=12	Present authors
A. tagala Cham.	2n=12	Present authors;
	2n=14	Gregory (1956), Ma (1989)
A. zollingeriana Miq.	2n=12	Peng *et al.* (1986)
Sect. *Gymnolobus* Duchartre		
Subsect. *Hexandrae* Duchartre		
Ser. *Thyrsicae* F. Gonzalez		
A. maxima L.	2n=16	Present authors
A. ovalifolia Duchartre	2n=16	Present authors
Ser. *Hexandrae*		
A. arcuata Mast.	2n=14	Gregory (1956), Present authors
A. argentina Griseb.	2n=14	Present authors
A. brasiliensis Mart. et Zucc.	2n=14	Gregory (1956)
A. cymbifera Mart. & Zucc.	2n=14	Gregory (1956)
var. *abbreviata* Duchartre	2n=14	Present authors
A. elegans Mast.	2n=14	Gregory (1956), Yu *et al.* (1995)
A. fimbriata Cham. & Schltdl.	2n=14	Gregory (1956), Present authors
A. giberti Hook. var. *paulistana* Hoehne	2n=14	Gregory (1956)
A. gigantea Mart. et Zucc.	2n=14	Gregory (1956)
A. grandiflora Sw.	2n=14	Gregory (1956)
A. hians Willd.	2n=14	Gregory (1956)
A. macroura Duchartre	2n=14	Moore (1970), Present authors
A. odoratissima L.	2n=14	Present authors
A. ringens Vahl	2n=14	Gregory (1956), Present authors
	2n=12	Yu *et al.* (1995)
A. triangularis Cham.	2n=14	Present authors
A. weddellii Duchartre		
var. *rondoniana* Hoehne	2n=14	Gregory (1956)
Subsect. *Pentandrae* Duchartre		
A. micrantha Duchartre	2n=14	Present authors

just clarified from the molecular phylogeny (Murata *et al.* 2001, see also Fig. 2). In earlier cytological studies the subgenus *Siphisia* had been defined as a group with the number $2n=28$ (Ma 1989, 1990), based on the fact that several North American species representing the subgenus commonly had the number $2n=28$ (Gregory 1956). Nevertheless our results and the more current information (Sugawara & Murata 1992, Yu *et al.* 1995) indicate that the number

$2n=32$ is common to all the species examined in the subgenus *Siphisia*. Thus the subgenus *Siphisia* may be characterized by the number $2n=32$. We are doubtful about the number $2n=28$ found in several species of subgenus *Siphisa* (see Fig. 2). To assess and validate the cytological features of this subgenus further investigations are needed on North American species.

The subgenus *Aristolochia* is morphologically characterized by having an apically dehiscent capsule, a bi-lobed or undivided perianth-tube and a penta- or hexa-lobed gynostemium (Gonzalez 1999). Despite sharing such floral features the subgenus has been further subdivided into two sections, four subsections and two series (Gonzalez 1999). Monophyly of subgenus *Aristolochia*, subsections *Aristolochia* and *Podanthemum*, and series *Thyrsicae* is strongly supported by the molecular data (Fig. 2). In contrast, two sections *Aristolochia* and *Gymnolobus* are not monophyletic (Fig. 2). As shown in Figure 2, the subgenus *Aristolochia* is evolutionary diversified in chromosome number and indicates three different numbers, $2n=12$, 14 and 16. Among three different numbers, the number $2n=14$ is more commonly found in species of the subgenus, and appears to have occurred parallel in three subsections of the subgenus *Aristolochia*. The subsection *Podanthemum* seems to be characterized by the number $2n=12$, although a heterogeneous chromosome number ($2n=14$) has been reported for *A. tagala*. It is also suggested that the number $2n=16$ is characteristic to series *Thyrsicae* including *A. maxima* and *A. ovalifolia*.

As already being stated by Murata *et al.* (2001), the evolutionary change of chromosome numbers within the genus *Aristolochia* is a very interesting problem. However, our information on the chromosome numbers of *Aristolochia* is still insufficient in discussing the above problem. Table 2 shows the chromosome numbers of 68 taxa in *Aristolochia* reported so far. The number $2n=28$ reported for several species in the subgenus *Siphisia* is doubtful, based on the current (Sugawara & Murata 1992, Yu *et al.* 1995) and present studies. In addition, the subsection *Aristolochia* appears to have been more remarkably diversified in chromosome number than we expect, since it represents several different numbers, such as $2n=8$, 10, 26 and 36 (see Table 2). In order to clarify the above problem and to discuss the evolutionary change of chromosome numbers a more detailed and comprehensive survey on chromosomes and a molecular phylogeny in the genus *Aristolochia* is needed.

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References

Fedrov, A. A. 1969. Chromosome Numbers of Flowering Plants. Acad. Sci. U.S.S.R., Leningrad.

Goldblatt, P. 1981. Index to Plant Chromosome Numbers for 1975-1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5: 1-553.

——— & D.E. Johnson. 1990. Index to Plant Chromosome Numbers for 1986-1987. Monogr. Syst. Bot. Missouri Bot. Gard. 30:1-243.

——— & ———. 1991. Index to Plant Chromosome Numbers for 1988-1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40: 1-238.

——— & ———. 1996. Index to Plant Chromosome Numbers for 1992-1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58: 1-276.

Gonzalez, F. 1999. Inflorescence morphology and the systematics of Aristolochiaceae. Syst. Geogr. Pl. 68: 159-172.

—— & D. W. Stevenson. 2000. Perianth development and systematics of *Aristolochia*. Flora 195: 370-391.

Gregory, M. P. 1956. A phyletic rearrangement in the Aristolochiaceae. Amer. J. Bot. 43: 110-122.

Levan, A., K. Fregda & A. A. Sandberg. 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52: 201-220.

Ma, J.S. 1989. A revision of *Aristolochia* Linn. from E. & S. Asia. Acta Phytotax. Sin. 27: 321-364 (In Chinese with English summary).

——. 1990 The geographical distribution and the system of Aristolochiaceae. Acta Phytotax. Sin. 28: 345-355 (In Chinese with English summary).

Moore, R. J. 1970. Index to Plant Chromosome Numbers for 1968. International Bureau for Plant Taxonomy and Nomenclature of the International Association for Plant Taxonomy, Utrecht.

——. 1977. Index to Plant Chromosome Numbers for 1973-1974. International Organization of Plant Biosystematists, Utrecht.

Murata, J., T. Ohwi, S.G. Wu, D. Darnaedi, T. Sugawara, T. Nakanishi & H. Murata. 2001. Molecular phylogeny of *Aristolochia* Aristolochiaceae) inferred from matK sequences. Acta Phytotax. Geobot. 52: 75-83.

Nardi, E. 1984. The genus *Aristolochia* L. (Aristolochiaceae) in Italy. Webbia 38:221-300.

Ornduff, R. 1967. Index to Plant Chromosome Numbers for 1965. International Association for Plant Taxonomy and Nomenclature, Utrecht.

Peng, C. I., S.F. Yen & J.Y. Guo. 1986. Notes on the chromosome cytology of some rare, threatened, or endangered plants of Taiwan (I). Bot. Bull. Acad. Sinica 27: 219-235.

Pfeifer, H.W. 1966. Revision of the north and central American hexandrous species of *Aristolochia* (Aristolochiaceae). Ann. Missouri Bot. Gard. 53: 115-196.

Schmidt, O.C. 1935. Aristolochiaceae. *In*: Engler, A. & K. Prantl (eds.), Die Naturlichen Pflanzenfamilien, 2-Aufl. 16: 204-242.

Sugawara, T. & J. Murata. 1992. Chromosome numbers of eight species of *Aristolochia* (Aristolochiaceae) from East Asia. Acta Phytotax. Geobot. 43: 27-30.

Yu, W., L.-Q. Huang & C.-Y. Cheng. 1995. A preliminary cytotaxonomical study on the Chinese *Aristolochia* (Aristolochiaceae). Bull. Bot. Res. Harbin 15: 199-205 (In Chinese with English summary).

APPENDIX 1. Voucher information and GenBank accession number on seven species of *Aristolochia* added newly to the molecular phylogenetic tree by Murata *et al.* (2001).

Species	GenBank accession no.	Collection data	Voucher no.
Aristolochia brasiliensis Mart. et Zucc.	AB071819	cult. in Setsunan Univ.	SETS82
Aristolochia californica Torr.	AB071813	cult. in Setsunan Univ.	SETS76
Aristolochia grandiflora Sw.	AB071812	cult. in Setsunan Univ.	SETS75
Aristolochia micrantha Duchartre	AB071815	Mexico, cult. in Setsunan Univ.	SETS78
Aristolochia tomentosa Sims	AB071814	cult. in Setsunan Univ.	SETS77
Aristolochia zollingeriana Miq.	AB071817	cult. in Setsunan Univ.	SETS80
Aristolochia sp. JM03	AB071818	cult. in Setsunan Univ.	SETS81

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